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Award Number: W81XWH-06-1-0673

TITLE: ErbB2 Trafficking and Signaling in Human Vestibular Schwannomas

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REPORT DATE: October 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
01-10-2007	Annual	15 Sep 2006 – 14 Sep 2007

4. TITLE AND SUBTITLE

ErbB2 Trafficking and Signaling in Human Vestibular Schwannomas

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0673

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Iowa
Iowa City, IA 52242

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Not Provided

15. SUBJECT TERMS
Not Provided

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	5	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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Annual Report Award W81XWH-06-1-0673 **November 22, 2007**
Erb2 trafficking and signaling in human vestibular schwannomas
Marlan R. Hansen

During the first year of the award we have made substantial progress in achieving the aims of the proposal. I will discuss the progress for each aim of the proposal.

Specific aim 1. Determine the ability of merlin to regulate ErbB2 localization and activity in vestibular schwannoma (VS) cells. Until recently we were unable to use human vestibular schwannoma (VS) specimens while we were working to obtain Human Subjects approval. During this period, we focused on correlating the status of merlin phosphorylation with ErbB2 trafficking in normal Schwann cells (SCs). First, we determined that the trafficking of ErbB2 into lipid rafts in SCs correlates with loss of axonal contact, phosphorylation of merlin on Serine 518, and proliferation (Figs 1 and 2). Thus, phosphorylation of merlin on Serine 518 (S518), which inhibits its growth suppressive function, is correlated with the movement of ErbB2 into lipid rafts in the cell membrane. We have previously shown that ErbB2 constitutively resides in lipid rafts in human vestibular schwannoma cells that lack functional merlin (Brown and Hansen, Otology & Neurotology, in press). Since we did not initially have approval to work with human VS specimens, we worked on subcloning various merlin constructs, including those with S518 mutations, into adenoviral vectors. This will allow us to directly test the role of merlin in regulating the trafficking of ErbB2 in human vestibular schwannoma cells. Recently the Dept. of Defense and the Univ. of Iowa concurred that the project did not require IRB approval since it did not qualify as Human Subjects research and in the coming year we will be able to determine the extent to which replacement of merlin in human vestibular schwannoma cells regulates the trafficking of ErbB2 within the cell membrane.

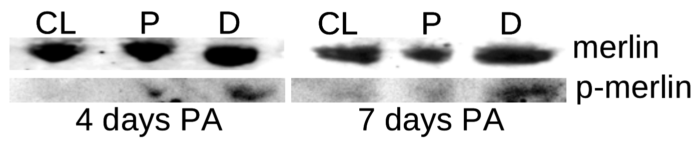

Figure 1. Sectioning of rat sciatic nerve results in increased merlin S518 phosphorylation (**p-merlin**) in the distal (**D**) segment compared to the proximal (**P**) segment and contralateral nerve (**CL**)

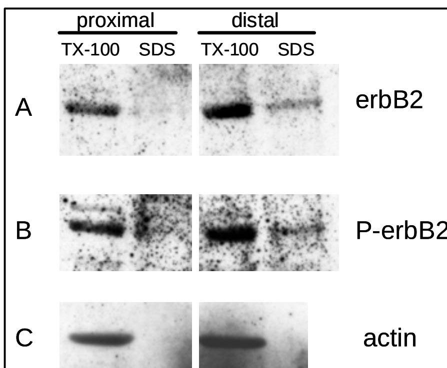

Figure 2. ErbB2 moves into the Triton-X-100 insoluble (lipid raft) fraction and becomes phosphorylated (p-ErbB2) in denervated Schwann cells (distal) following axotomy.

Specific aim 2. Determine whether phosphorylation of merlin on serine 518 (S518) by protein kinase A (PKA) inhibits merlin's ability to regulate ErbB2 trafficking and suppress VS cell proliferation. Again here we were initially limited in the work we could do with human specimens. However, we have been able to demonstrate that activation of protein kinase A (PKA) with forskolin (FSK 5 μM) leads to

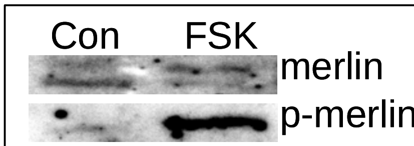

Figure 3. Treatment of cultured rat sciatic nerve Schwann cells with forskolin (FSK, 5 μM) increases merlin S518 phosphorylation relative to control (CON) cultures.

merlin S518 phosphorylation in rat SCs (Fig. 3) and that this correlates with proliferation and with movement of ErbB2 to the cell membrane. In the coming year, we will be able to directly test the role of S518 phosphorylation by PKA in regulating ErbB2 trafficking and VS proliferation.

Specific aim 3. Determine whether ErbB2 inhibitors potentiate the ability of radiation therapy (RT) to induce VS apoptosis and reduce proliferation. Following approval to proceed with the use of human tissue we have been able to perform radiation experiments on several cultured human VS specimens. We demonstrate that doses of ≥30 Gy are required to limit VS cell proliferation and that doses ≥40 Gy are required to induce apoptosis (Fig. 4). Furthermore, we show that inhibition of ErbB2, which reduces VS cell proliferation, protects VS cells against radiation induced cell death. Conversely, activation of ErbB2 by treatment with neuregulin, which promotes VS cell proliferation, increases the radiosensitivity of human VS cells. These results imply that (1) VS are radioresistant relative to most neoplasms, (2) the radiosensitivity of human VS cells depends on proliferative status, and (3) ErbB2 signaling sensitizes VS cells to radiation by promoting proliferation. Thus, we have been able to make substantial progress on the proposed experiments in this aim and are now exploring the possibility that persistent activation of c-Jun N-terminal kinase signaling in protects human VS cells from radiation induced cell death by limiting the accumulation of reactive oxygen species.

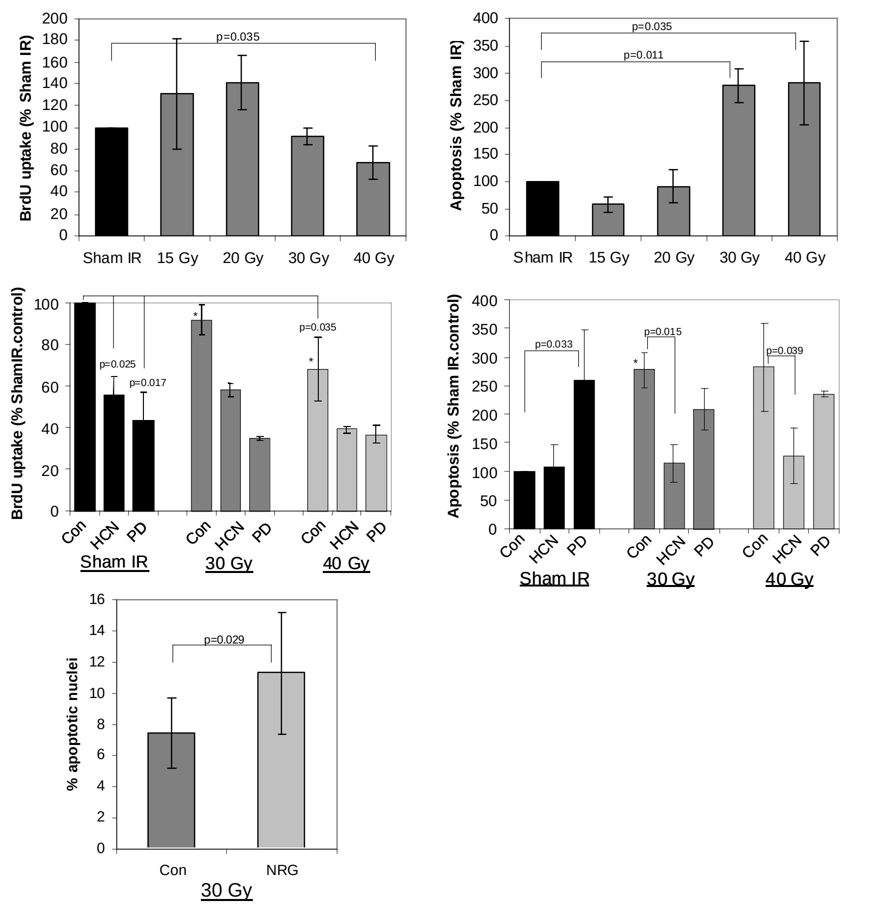

Figure 4. Effects of ErbB2 signaling on vestibular schwannoma radiosensitivity. Inhibition of ErbB2 with trastuzumab (Herceptin, HCN) or PD158980 reduced radiation induced VS cell apoptosis, while neuregulin (NRG), increased VS cell radiosensitivity.